THE ANNALS

AND

MAGAZINE OF NATURAL HISTORY.

[FIFTH SERIES.]

"................ per litora spargite muscum,
Naiades, et circùm vitreos considite fontes:
Pollice virgineo teneros hìc carpite flores:
Floribus et pictum, divæ, replete canistrum.
At vos, o Nymphæ Craterides, ite sub undas ;
Ite, recurvato variata corallia trunco
Vellite muscosis e rupibus, et mihi conchas
Ferte, Deæ pelagi, et pingui conchylia succo."
N. Parthenii Giannettasii Ecl. 1.

No. 115. JULY 1887.

I.—*The Significance of the Yolk in the Eggs of Osseous Fishes.* By EDWARD E. PRINCE, St. Andrews Marine Laboratory.

[Plate II.]

MUCH has been recently written upon the relation of the food-yolk and the germ in Teleostean eggs, yet little unanimity seems to characterize the conclusions reached by various observers. It is generally allowed that the free margin of the thickened blastodermic ring is really the lip of the blastopore—the entire periphery being so, and not merely, as Mr. Cunningham has ably shown *, an invaginated arc, as in the Elasmobranchs. The difference of opinion that exists arises, however, from the various views held as to the nature of the yolk and its function during development. Häckel, from his study of a pelagic ovum, concluded that the yolk in Teleostean eggs was emphatically distinct from the germ †, a contrast in the main constituents of the egg that M. Coste seems to have first truly signalized ‡. Later investigators (Klein,

* Quart. Journ. Microsc. Sci., Nov. 1885.
† Jenaische Zeitschr. vol. ix. 1875.
‡ Gazette médic. de Paris,' No. 17, 1855, p. 257.

Kingsley and Conn, and others) have adopted this view, according to which the egg of an osseous fish is, perhaps, one of the most marked examples of the meroblastic type.

In the Mammalian ovum we know that there is no such broad distinction; but, as in *Amphioxus*, the yolk that is present and the active protoplasm are so intermingled that segmentation is complete. The Amphibian ovum—*Rana*, for example, is also holoblastic; but the yolk so preponderates towards the vegetal pole that the cleavage-furrows, beginning at the opposite or animal pole, progress with increasing difficulty as they approach the former region. The animal pole in the Amphibian egg is distinguished by the great abundance of active protoplasm and the minute size of the suspended yolk-spherules, as well as its more rapid cleavage. Still more marked is this bipolar segregation in the Sauropsidan and Elasmobranch ovum; but in the Teleostean egg it is most complete—a distinctly marked germinal disk, composed almost entirely of clear protoplasm, being formed by the withdrawal of germinal matter from the granular yolk. The separation may be very apparent, even before fertilization, in certain Teleosteans—a *discus proligerus* collecting, similar to the superficial protoplasmic disk seen lying upon the yellow food-yolk in the mature Selachian ovum.

Usually both constituents are so intermingled as to be undistinguishable in the living egg until a period of one or two hours has elapsed after the entrance of the spermatozoon, when the translucent homogeneous blastodisk is rapidly outlined at the animal pole, either at the upper or the lower side of the egg, according to the species *. The separation of germinal matter from the food-yolk is carried to such a degree in the Teleostean ovum that it presents a marked contrast to the type of egg seen in the bird or shark, and still more in the frog or lamprey (compare figs. 1 and 2, Pl. II.). E. van Beneden, in his classical memoir "Sur la composition et la signification de l'œuf" †, speaks of the nutritive part as deutoplasm, and lays stress on its non-integral or accessory nature, on its purely passive function, and on the fact that in some eggs it is absent, though when it is present it serves to nourish the blastoderm and embryo. This contrast between the deutoplasm and the germinal protoplasm is illustrated in a marked degree in the Teleostean ovum, yet the existence in it

* In the Salmonidæ the germ surmounts the upper pole of the egg, whereas in the ova of the Pleuronectidæ and Gadidæ it is formed at the inferior pole.

† E. van Beneden, Mém. Cour. l'Acad. Roy. de Belgique, tome xxxiv. 1870.

of an extra-blastodermic layer of protoplasm (figs. 1, 9, and 10, *c.p.* and *perib.*, Pl. II.) must not be ignored. The very fact, however, that such an area or periblastic ring exists supports the view here propounded. If the protoplasm interfused amongst the yolk becomes, by a physical process of separation and superficial transference, concentrated at the animal pole, as represented in the diagram fig. 9, Pl. II., it is easy to see that some of it may be left at the margin as a peripheral ring. The process is slow, and much protoplasm may continue to pass towards the animal pole, even after the germinal disk is defined and segmentation is in progress. Such, in fact, is the case, and this is the explanation of the extra-germinal area, appropriately called periblast. Mr. G. Brook aptly expressed the condition of this area when he said * that the germinal protoplasm is for the most part included in the first two cells of the blastodisk, and, "as if not to waste any material, the remainder collects around this disk and is afterwards developed into the periblast." Further away from the disk the periblast (figs. 9 and 10, *perib.*, Pl. II.) thins out and gradually passes into a filmy protoplasmic layer, uniformly investing the remaining surface of the yolk and known as the cortical layer (Pl. II. figs. 9 and 10, *perib.*).

Kingsley and Conn affirm † that in the earliest stages the periblast is not present; and, paradoxical as it may seem, they are right, for the periblast, as such, does not exist until a later period—until, in fact, the limits of the disk are indicated with some precision by the progress of segmentation (compare figs. 1 and 9, Pl. II.). The protoplasmic cortex, of which the periblast forms merely a thickened annular portion, is really present from the moment that superficial segregation begins, and so long as the process continues the cortical layer persists, and even in advanced embryos it is distinguishable, passing beneath the embryonic trunk, between the hypoblast and the remnant of the yolk (Pl. II. fig. 11, *c.p.*). Segregation is not only superficial but, as stated elsewhere ‡, there is also a subgerminal transference, and Mr. Brook has shown § that in *Clupea* these deep-seated tracts form definite ramifications amongst the yolk. The periblast is simply germinal matter which has not yet entered the disk, and that it gradu-

* Quart. Journ. Microsc. Sci., Jan. 1885, p. 4.

† Mem. Boston Soc. Nat. Hist., April 1883, p. 202.

‡ "Develop. of the Food-Fishes," Ann. & Mag. Nat. Hist. 1886, vol. xvii. p 447.

§ 'Fourth Annual Report of Fishery Board for Scotland,' 1885, App. F, no. i. pp. 34, 35.

ates into the yolk below is not surprising, for its protoplasm is continually *in transitû*.

Now the yolk in the Amphibian ovum becomes divided by cleavage into large nucleated yolk-cells, just as in the egg of *Petromyzon* (Pl. II. fig. 2, *y*), and enters more or less intimately into the formation of the embryo. The ventral lining of the mesenteron is really yolk-hypoblast, and arises directly from the yolk-cells proper, as Mr. Shipley shows in *Petromyzon*: the dorsal wall "is composed of columnar cells resembling those of the general epiblast; the cells forming the floor have the same characters as the yolk-cells" * (Pl. II. fig. 7, *y*). Nothing like this is seen in the Teleostean egg, though Mr. Brook, relinquishing the view referred to on a prior page, has adopted the conception that Teleostean and Amphibian ova are similar even in the details of their development "the derivatives of the animal and vegetative poles are in both cases practically identical." If the mesenteron in Osseous Fishes does not arise as a slit in the thickened median hypoblast, as the greater part of it really seems to do, but is largely built up out of nucleated periblast, as Mr. Cunningham has suggested †, the yolk is still not directly concerned in the process, the periblast being, as Klein says, a continuation of the germ, both are "one and the same substance" ‡. Kupffer's vesicle, which arises as a sub-embryonic chamber, is not ventrally limited by the yolk, but by the periblast. Throughout the embryonic period in Teleosteans the periblast intervenes as a continuous layer between the yolk and the germ (as shown in Pl. II. figs. 7 and 11, *c.p.*). Oellacher speaks of the germ as feeding on the yolk §, and Kingsley and Conn say that particles of yolk seem to be taken in after segmentation has begun ||, while Klein expresses the view, which Mr. Brook adopts, that the periblast performs the digestive function, so that, as the last-named author says, "large masses of yolk are incorporated within its substance and assimilated"¶. The formation of the disk and early protoplasmic cortex is due, it is granted, to a kind of physical transference, mainly superficial segregation: At what point, it may be asked, does such segregation cease and digestion begin? No such point can be determined. The yolk, in fact, does not diminish to such an extent as the theory of digestion *plus* segregation would imply, as we see by comparing the

* Quart. Journ. Microsc. Sci., Jan. 1887, p. 329.
† *Ibid.*, Jan. 1885, p. 7, and Nov. 1885, pp. 20, 21.
‡ *Ibid.* vol. xvi. 1876, p. 118.
§ Zeitschr. f. Wiss. Zool. Bd. xxii. p. 4. || *Loc. cit.* p. 127.
¶ 'Report of Fishery Board for Scotland,' 1885, p. 35.

bulk of the yolk in the early ovum (Pl. II. fig. 1, *y*) and in a later stage when the embryo is fairly advanced, as in Pl. II. fig. 3, *y*, and the very slight diminution that does occur (*vide* Pl. II. fig. 4, *y*) can be accounted for by the continued separation of the interfused protoplasm. The large size of the yolk-mass, in the emerged embryos of pelagic and demersal forms alike, indicates that any very active process of digestion is doubtful. That the globular ball of yolk is not an integral part of the germ or embryo is sufficiently shown by the ease with which it can be removed from its periblastic and embryonic envelopes in hardened specimens. The yolk seems to be chiefly utilized during the early stages of the active liberated embryo, diminishing greatly during the first fortnight after hatching (compare figs. 4 and 6, *y*, Pl. II.), and in those species which develop a vitelline circulation the rapid removal of the yolk-granules can be readily understood. In pelagic forms, without such vascular provision, the yolk is less rapidly used up; and, doubtless, in these the cœliac and hepatic blood-vessels, being in close proximity to the yolk-surface, effect the absorption.

All this evidences the accessory nature of the yolk in Teleosteans. It is an appendage—a cænogenetic addition or adaptation, as Häckel regarded it—not directly contributing to the building up of the tissues, but mainly serving to furnish pabulum to the delicate and rudimentary embryo on emerging from the egg It is not more essentially connected with the development of the germ than the egg-envelope *. In hardened preparations it shows a granular structure, and when physically manipulated often has the texture of dense cork; and in the young salmon, as Professor M'Intosh long ago described, the yolk becomes less fluid, and by-and-by springs from the touch of a glass rod like a rounded and smooth bit of cartilage on simply transferring the embryo from fresh to salt water †. In the living egg it is a clear albuminoid matrix of the consistency of syrup, readily issuing from a puncture in the yolk-sac (Pl. II. fig. 5, *y*), and containing minute vesicles and refrangible particles, with the addition, in certain species, of large oleaginous spheres. The presence of these spheres in the yolk adds strength to the view that it is a nutritive appendix, for, as shown in a pre-

* *Vide* Quart. Journ. Microsc. Sci. vol. xvi. 1876.—Note on p. 56, where Prof. Ray Lankester distinguishes the added food-material and egg-envelopes as "matrificial" and not "ovificial" elements, like the protoplasm of the egg-cell proper.

† Quart. Journ. Microsc. Sci. vol. viii. 1868, p. 153.

vious paper *, the globules in question seem to have no intimate connexion with development, and are best regarded as redundant and probably ancestral elements, still persisting, but not immediately utilized by the germ.

If this view be correct, that the yolk is a trophic appendage, consisting in the later stages almost purely of inert nutritive matter, that the germ is discoblastic and becomes a discogastrula when the germinal cavity appears beneath it (Pl. II. fig. 10, *g.c.*), and hence that the invaginated rim represents the primitive enteric involution, like the inflected arc in Elasmobranchs and Amphibians, then the interpretation of the features presented by the Teleostean ovum becomes greatly simplified. Balfour speaks of such a mass of unsegmented yolk as corresponding to the large cells of the vegetal pole in a blastosphere; and E. van Beneden similarly regarded the deutoplasmic globe in a pelagic Teleostean ovum as a large endodermic cell, with a constitution analogous to a fat-cell †, a view shared by Hoffman and others. But the Teleostean germ never forms a blastosphere, with a more or less centrally situated segmentation-cavity or blastocœl, in addition to the large subgerminal chamber, which is always present at some stage. Van Bambeke alone amongst observers really describes a blastocœl in the egg of an osseous fish; but Oellacher, Kingsley and Conn, and other authors regard such an intrablastodermic cavity as an artificial product, and not a normal feature. The sub-blastodermic cavity present in the Teleostean ovum (Pl. II. fig. 10, *g.c.*) must be the homologue not of the Amphibian and Selachian segmentation-cavity, so-called, but of the enteric cavity, whose external opening is the blastopore. The germ, thus separated by a germinal cavity from the yolk, consists of two lamellæ, ectoderm and primitive endoderm, like a two-layered gastrula; the external layer or epiblast appears to be one cell in thickness; but the endoderm, or "lower layer," consists of several layers of cells (Pl. II. fig. 10, *g*). From its mouth or blastopore the yolk forms an enormous protruding mass, an exaggeration of the yolk-plug which fills up the anus of Rusconi in *Rana* (Pl. II fig. 10, *y*).

The important feature in the Teleostean egg is not the fact that the yolk is stored away at one pole of the egg, for the egg of the Amphibian or Cyclostome may be described as simply the ovum of *Amphioxus* with a large amount of trophic matter stored away in its lower part, nor that the yolk-cells

* "On the Presence of Oleaginous Spheres in the Yolk of Teleostean Ova," Ann. & Mag. Nat. Hist., Aug. 1886.

† Quart. Journ. Microsc. Sci. vol. xviii. 1878, p. 52.

are broken down and form a syncytium, but that the germinal matter is so concentrated at one pole as to have little more connexion with the yolk than that of juxtaposition. The yolk seems to have no essential *rôle* in segmentation, but is an appendage to the early germ as to the later embryo. The nature and function of the periblast and cortical protoplasm need not be dwelt upon; they are continuous with and form part and parcel of the germ. The origin and fate of the nuclei which appear in them is by no means decided. As Klein declared, they are not identical with the yolk nuclei of the Elasmobranch egg *, and they probably originate, as Agassiz and Whitman hold, and as Wenckebach's recent researches tend to show †, in the segmented blastoderm itself. We know how greatly the food-yolk, when it crowds segmenting cells, alters their character and disposition; and the possibility seems naturally to follow that when, as in the Teleostean egg, the yolk becomes almost wholly separated from the germ, a less distorted and more primitive condition may be resumed. We can thus understand how, notwithstanding the great bulk of the yolk, the blastopore in Osseous Fishes is symmetrical, and coincides with the entire inflected margin of the germ, while the germ itself forms, not a blastosphere with a transient segmentation-cavity, as well as a permanent enteric invagination, but a concave two-layered gastrula, enclosing or rather arching over a primitive gastric chamber (Pl. II. fig. 10, *g.c.*). In this enteric chamber, roofed over by invaginated hypoblast and with a floor of periblast (Pl. II. fig. 10, *perib.*), the globe of passive yolk-matter (Pl. II. fig. 10, *y*) is seated, and projects from the blastopore until the free margin of the latter has so far progressed over its surface as to entirely envelop it. It persists in the perivisceral cavity as a ventral protuberance for some time after the embryo has emerged (Pl. II. fig. 6, *y*) until it is completely disintegrated and absorbed.

EXPLANATION OF PLATE II.

Fig. 1. Ovum of *Gadus æglefinus,* fifth hour; four blastomeres nearly completed. *bl.*, blastomeres; *c.p.*, cortical protoplasm passing to the animal pole; *y*, yolk.

Fig. 2. Ovum of *Petromyzon fluviatilis,* about same stage as fig. 1 (after Shipley), showing the yolk included in the segmentation process. *bl.*, blastomeres; *y*, yolk.

* Quart. Journ. Microsc. Sci. vol. xvi. 1876, p. 128.
† Archiv f. mikr. Anat. Bd. xxviii. 1886.

Fig. 3. Ovum of *G. æglefinus*, some time after closure of the blastopore; the embryo fairly advanced, but the yolk (*y*) shows very slight diminution.

Fig. 4. Ovum of *G. æglefinus*; embryo about to emerge from the egg-capsule, which is ruptured. The yolk (*y*) has diminished to some extent, and a perivitelline chamber intervenes between the yolk-surface and the embryonic membrane (*e.m.*).

Fig. 5. Emerged embryo of a Pleuronectid, species not known. Portion of the yolk seen protruding from an accidental rupture in the embryonic membrane (*e.m.*).

Fig. 6. Embryo of *Gadus æglefinus*, six days after hatching; yolk (*y*) still persisting, but showing very evident diminution.

Fig. 7. Transverse section through embryo of *Petromyzon* (after Shipley). Yolk-cells (*y*) entering actively into the formation of the embryonic tissues, especially the gut (*g*): *n*, notochord; *mes.*, mesoblast.

Fig. 8. Transverse section of *Gadus æglefinus*, about same stage as fig. 7. The yolk (*y*) is separated from the embryo by the cortical protoplasm (*c.p.*) and the hypoblast (*hyp.*), and does not directly form embryonic tissue. *n*, notochord; *mes.*, mesoblast.

Fig. 9. Diagram of Teleostean ovum when the periblast (*perib.*) is first clearly distinguishable. The radial arrows indicate the passage towards the surface of the protoplasm mingled with the yolk (*y*), and forming the cortical protoplasm (*c.p.*). *g*, germ.

Fig. 10. Diagram of Teleostean ovum at a later stage. No intra-blastodermic segmentation-cavity exists; but a germinal cavity (*g.c.*) exists, roofed over by the germ and floored by periblast (*perib.*).

Fig. 11. Transverse section of *G. æglefinus* on second day after hatching. The cortical protoplasm (*c. p.*) still separates the embryo from the yolk (*y*). The hypoblastic gut (*g*) is now fully formed and invested by a layer of mesoblast; its lumen is ciliated. *e.m.*, embryonic membrane formed of two layers, epiblast and hypoblast.

II.—*Notes on Coleoptera, with Descriptions of new Genera and Species.*—Part VI. By Francis P. Pascoe, F.L.S., &c.

[Plate I.]

List of Genera and Species.

COLYDIIDÆ.

Bothrideres impressus.

PTINIDÆ.

Anobiinæ.

Clada (*n. g.*) Waterhousei.

TELEPHORIDÆ.

Drilinæ.

Eugeusis nigripennis.
Selasia pulchra.
—— laticeps.